DEFINITIONS OF BACTERIAL OXYGEN RELATIONSHIPS¹

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A summary of the conditions to be considered by the roundtable was presented by Dr. Carl Lamanna. It was indicated that two entirely different situations should be covered by any definitions of bacterial oxygen relationships. The first of these is a description of the environment or atmosphere in which the bacteria can grow. This could be handled adequately with the terms aerobic and anaerobic. Aerobic would indicate the presence of air at atmospheric pressure, whereas anaerobic would apply to the absence of air. The second situation to be covered is the metabolic use of gaseous oxygen by living bacteria. Organisms capable of using oxygen would be defined as oxybiontic (oxybiotic)² and those unable to use oxygen as anoxybiontic (anoxybiotic).

Agreement was quite general on the suggested use of the terms aerobic and anaerobic. Mr. V. B. D. Skerman believed that the term aerobic should apply not only to air but also to any gas mixture containing 21 per cent by volume of oxygen. It would not be reasonable to set the air pressure required for aerobic growth because of

¹ A roundtable discussion on the oxygen relationships of bacteria was held on the evening of May 4, 1954, at the Society of American Bacteriologists meeting in Pittsburgh, Pennsylvania. Several persons had been invited to participate by Richard H. McBee, the convener. The majority of those invited to participate were present, as were several others who were interested in the topic.

² The terms oxybiotic and anoxybiotic were used by Weinland to define respiration with and without gaseous oxygen by Ascaris (Weinland, E. 1906 Über den anaeroben (anoxybiotischen) Abschnitt der intermediären chemischen Prozesse in der Puppen von Calliphora. Z. Biol., 48, 87-140). The change in spelling is that of later workers.

large differences in normal atmospheric pressure encountered at different elevations.

A discussion followed in which it was pointed out by Dr. H. W. Seeley and Dr. Norman D. Gary that the ability of an organism to utilize oxygen was often difficult to determine, and that some of the lactic acid bacteria which apparently did not use atmospheric oxygen to their advantage in their metabolism did contain enzymes which could transfer hydrogen to oxygen. The question was also raised as to how one would classify an organism that damaged itself by the production of hydrogen peroxide in the presence of gaseous oxygen. These situations were not resolved in the discussion. It was concluded, however, that the fact of oxygen utilization in itself should not be a basis for calling an organism aerobic.

Dr. L. S. McClung and Dr. L. DS. Smith believed that the terms discussed were not adequate to define some of the clostridia and other bacteria which have been called microaerophilic. Since the derivation of the term microaerophilic does not describe the situation encountered when clostridia grow aerobically without oxygen entering into cellular metabolism, it was suggested that a term other than microaerophilic be used to describe these organisms. The terms oxytolerant, oxysensible, and aerotolerant were proposed. Aerotolerant received the greatest support in the ensuing discussion.

Dr. N. R. Smith believed that it would be logical and possible to change the description in Bergey's Manual of some of the clostridia from microaerophilic to anaerobic, aerotolerant. Dr. Lamanna called attention to the fact that upon critical examination organisms called microaerophilic often were found to require for the initiation of growth, or optimal growth, greater than atmospheric concentrations of carbon di-

oxide rather than reduced oxygen pressure. The term microaerophilic would not seem truly descriptive for such organisms.

Dr. L. DS. Smith defended the term microaerophilic as being applicable to the organism *Vibrio fetus* and possibly to some members of the genus *Brucella*. It was generally agreed that microaerophilic might be retained as a descriptive term but that it should not be used loosely.

The widely-used term facultative was discussed at some length by Dr. Owen B. Weeks and Mr. Skerman. All the participants actively entered into the discussion. Dr. N. R. Smith proposed that facultatively anaerobic apply to those aerobic bacteria that can obtain their oxygen for growth from combined oxygen, such as nitrates, etc., in the absence of free oxygen. From the discussion, it was decided that the inclusion of materials other than free oxygen in the definitions would make them unnecessarily complicated. It was finally decided that the term facultatively anaerobic should be used to describe aerobic bacteria that have the ability to grow in the absence of atmospheric oxygen.

After a short summation period to insure that all of the points discussed were adequately defined, a group consisting of Drs. Lamanna, Weeks, and McBee was designated to summarize the discussion and if it seemed to serve a useful purpose, to publish the summary in the form of a recommendation.

The report of this group follows:

The terms aerobic, anaerobic, and facultatively anaerobic should be applied only to the description of practical cultural conditions used for the cultivation of bacteria or to the growth of bacteria under these conditions. Aerobic bacteria will grow on the surface of a simple solid medium exposed to air. Anaerobic bacteria will not grow on the surface of a simple solid medium freely exposed to air. Facultatively anaerobic bacteria are those aerobic bacteria which have the ability to grow anaerobically. The word facultative should not be used by itself.

These terms are usable only if they are applied to practical cultural conditions, that is, air refers to the atmosphere found in the laboratory of the investigator doing the work, and a simple solid medium would usually be one solidified with agar and containing those materials needed for growth but without the addition of reducing substances added for the sole and specific purpose of reducing the oxidation-reduction potential.

Growth beneath the surface of an agar medium or in a fluid medium should not be considered as being either aerobic or anaerobic, and these cultural conditions should not be used to establish oxygen relationships in the place of more precise studies.

The requirements for gaseous oxygen in the growth of an organism should be considered apart from the conditions of culture since they are not necessarily related. The term oxybiontic could be applied to those bacteria capable of using atmospheric oxygen in their growth, whereas anoxybiontic would apply to those bacteria not capable of using atmospheric oxygen in their growth. Many bacteria have not been studied adequately to permit their classification on the basis of oxygen utilization. It should, however, be included in the description of each new species and in studies which involve re-examination of a taxonomic group of bacteria.

Cultural and metabolic situations have been encountered that are not adequately covered. These definitions should, however, help to clarify the description of most bacterial species. The exceptions will require more detailed descriptions. For example, the clostridia which will grow to a limited extent under aerobic conditions should probably be defined as aerotolerant anaerobic anoxybiontic rather than as aerobic or microaerophilic. A full description would necessarily contain the explanation that limited growth would occur under aerobic conditions. The term microaerophilic should be retained for those bacteria which may be found to be truly microaerophilic, i.e., incapable of growth either under aerobic conditions or in the complete absence of gaseous oxygen.

In the case of so-called microaerophilic organisms actually requiring increased carbon dioxide tensions rather than reduced oxygen tensions for growth, the term microaerophilic should not be used. Consideration should be given to the word capneic to describe these organisms. To our knowledge capneic was introduced by Rose³ to describe incubators designed for the incubation of bacterial cultures in atmospheres of controlled carbon dioxide content.

The terms which have been defined may be used to describe cultural habits as well as metabolism without confusion. Thus Pseudomonas aeruginosa is aerobic with an oxybiontic metabolism; Escherichia coli is facultatively anaerobic with an oxybiontic metabolism, Streptococcus lactis is

³ Rose, S. B. 1942 The importance of CO₂ in diagnostic bacteriology with observations on a CO₂ (cpaneic) incubator. Am. J. Clin. Pathol., 12, 424-433.

facultatively anaerobic with an anoxybiontic metabolism, Clostridium histolyticum is anaerobic, aerotolerant with an anoxybiontic metabolism; and Clostridium tetani is anaerobic with an anoxybiontic metabolism. This could be presented for descriptive purposes as

Oxygen relationships:

Cultural—aerobic, facultatively anaerobic, anaerobic, or aerotolerant anaerobic; possibly microaerophilic or capneic.

Metabolism—oxybiontic, anoxybiontic, or not known.